THE PRESENCE OF A CIRCULATING ANTI-COAGULANT IN A MALE MEMBER OF A HEMOPHILIAC FAMILY.

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The theoretical possibility that anti-coagulants might be present in the circulating blood has been considered from time to time. A comprehensive discussion of this subject has been given recently by Quick.[1] He has shown that there is "good evidence that heparin or a closely related substance appears in the blood in peptone and in anaphylactic shock." He has concluded his discussion with a statement to the effect that it seemed questionable whether any anti-coagulant other than heparin needed to be considered as a cause of hemorrhagic conditions encountered clinically. However, Lozner, Jolliffe and Taylor[3] have reported the presence of a circulating anti-coagulant, which was considered not to be heparin, in a 61-year-old colored man. This patient was shown not to have hemophilia and these investigators stated that such a finding in true hemophilia was untenable.

The present report deals with the finding of such an anti-coagulant in a patient who had the triad, as given by Quick,[5] by which hemophilia is recognized, viz. inheritance through the female, occurrence only in the male, and a prolonged clotting time. In addition, this patient has shown the typical reaction of hemophilia as determined by the clotting time of recalcified plasma (Quick).[5]

Case Report.

W. P., No. 27899. This 44-year-old unmarried male has been studied on repeated occasions in the Rochester Municipal and Strong Memorial Hospitals since his first admission on September 19, 1929. The pertinent points in his story prior to his first admission to the hospital may be summarized as follows: At the age of 3 years, he bled

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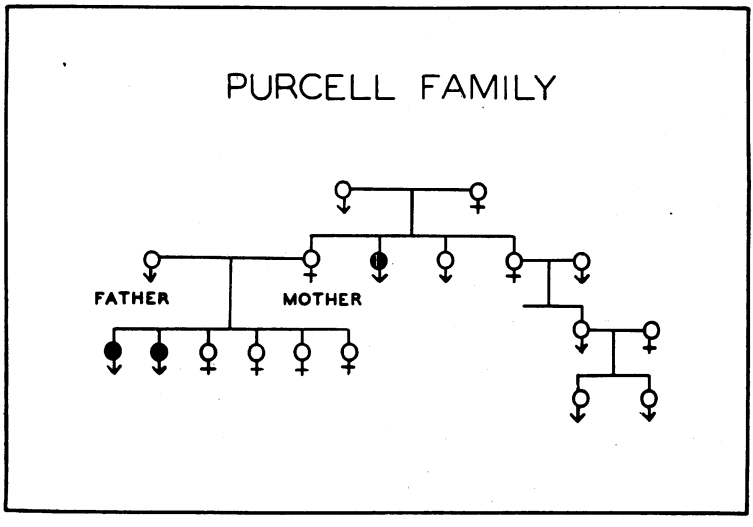


for 22 days following an incision of his lip. At 6 years of age, he was thought to have had blood in his urine. Up to the age of 15, his gums oozed blood at frequent intervals. For 16 years prior to his first admission, he had had sporadic pain and swelling in the elbows and knees with subsequent stiffening of these joints. He had always bled for prolonged intervals from small incisions. He had had several extractions of teeth with subsequent bleeding and he had, also, had many spontaneous giant ecchymoses. He had a brother with a typical picture of hemophilia and one maternal uncle had died from bleeding following an incision. His first admission to this clinic was for bleeding following extraction of a tooth. He has been admitted to the hospital on 18 occasions since that time. Two of these admissions were due to bleeding from his teeth. Nine of the admissions were due to hematuria. The other major complaints during this period of study have been gastro-intestinal bleeding, which has been present at the time of four admissions, hemoptysis, which has been present on one occasion, and hemorrhages into his joints, which have been present on seven admissions. He has received many transfusions. For the most part, his red blood cell, hemoglobin and white blood cell values have been within normal limits except, of course, after massive hemorrhage. Blood platelets have always been within normal limits. A

Table 1

W. P. Unit # 27899

Effect of Normal Blood or Plasma on the Coagulation Time of Patient's Blood in vivo.

Date	Substance Used	Coagulation Time in Minutes	
		Before Transfusion	After Transfusion
10-17-39	Blood - 200 c.c.	103	100
10-18-39	Blood - 250 c.c.	86	75
11-20-39	Blood - 200 c.c.	105	115
11-17-40	Fresh Plasma - 200 c.c.	75	60
4-24-41	Blood - 500 c.c.	89	70
6-2-41	Fresh Plasma - 220 c.c.	86	70

platelet count made on September 25, 1941, revealed 240,000 per cu. mm. His coagulation time has varied from 12 hours to 70 minutes but, in recent years when a standardized technic has been used, most of his values for coagulation time have been below 2 hours. The clots have always been normal and the bleeding time has been within normal limits. Prothrombin time and the values for calcium and fibrinogen of the blood have been normal repeatedly. Vitamin C content of the blood has been on two occasions slightly below normal. The only positive physical signs of any importance have been bleeding from his gums, dental caries and hemorrhage, swelling and ankylosis of the joints.

Experimental Studies with Reference to Coagulation Defect of Patient's Blood.

Repeated observations were made as to the effect of normal blood and plasma in vivo on the coagulation time of the patient's blood as shown in Table 1. It is seen that, while there was some diminution of the coagulation time of the patient's blood by transfusion of blood or plasma, this diminution was slight and by no means as great as that which ordinarily is seen in the case of patients with typical hemophilia.

Table 2

W. P. Unit # 27899

Effect of Oxalated Plasma of Patient on Purified Thrombin.

	Plasma in c.c.	Freshly Prepared Thrombin Solution in c.c.	Coagulation Time in seconds
Patient	1.0	0.5	85
Patient	1.0	0.5	94
Normal Control	1.0	0.5	95
Normal Control	1.0	0.5	87
Normal Control	1.0	0.5	85
Normal Control	1.0	0.5	80

Note that the coagulation time is the same with patient's plasma as with plasma of normal subjects.

Then, the effect of transfusion of blood of this patient to a patient with true hemophilia was studied. Prior to transfusion, the coagulation time of the patient's blood was 130 minutes and that of the recipient's (a true hemophiliac) blood was 100 minutes. Three hundred fifty c.c. of citrated blood was given. About 3 hours after the transfusion, the recipient began to bleed *continuously* from the vena puncture wound. On the following morning, the recipient's coagulation time was 158 minutes, which was the longest coagulation time that had been recorded on this patient on repeated previous observations. The bleeding continued throughout the night and until the following evening. Forty-eight hours after the transfusion, the recipient's coagulation time was 110 minutes and the bleeding had ceased.

Following these in vivo experiments, various studies of the patient's blood were made in vitro.

First, the effect of normal citrated blood on the coagulation time of the patient's blood was studied. Chart 1 shows that normal citrated blood had only a minimal effect on the coagulation time of the patient's blood, whereas it had a marked effect on the coagulation time of blood of a true hemophiliac. This experiment was repeated three times with the same results in each instance.

Second, the effect of the patient's blood on the coagulation time

Table 3

W. P. Unit # 27899

Effect of Lyophiled Plasma and Ether Extracted Plasma of Patient on Normal Blood.

Tube	Redissolved Plasma in c.c.	Redissolved Ether Extracted Dried Plasma in c.c.	Normal Saline in c.c.	Coagulation Time	Normal Whole Blood in c.c.
1	0.00	0.00	0.00	9	2.00
2	0.00	0.00	0.50	9	1.50
3	0.50	0.00	0.00	47	1.50
4	0.50	0.00	0.00	47	1.50
5	0.50	0.00	0.00	23	1.50
6	0.00	0.50	0.00	39	1.50
7	0.00	0.50	0.00	47	1.50
8	0.00	0.50	0.00	47	1.50
9	0.25	0.00	0.00	10	1.75
10	0.25	0.00	0.00	8	1.75
11	0.00	0.25	0.00	20	1.75
12	0.00	0.25	0.00	23	1.75

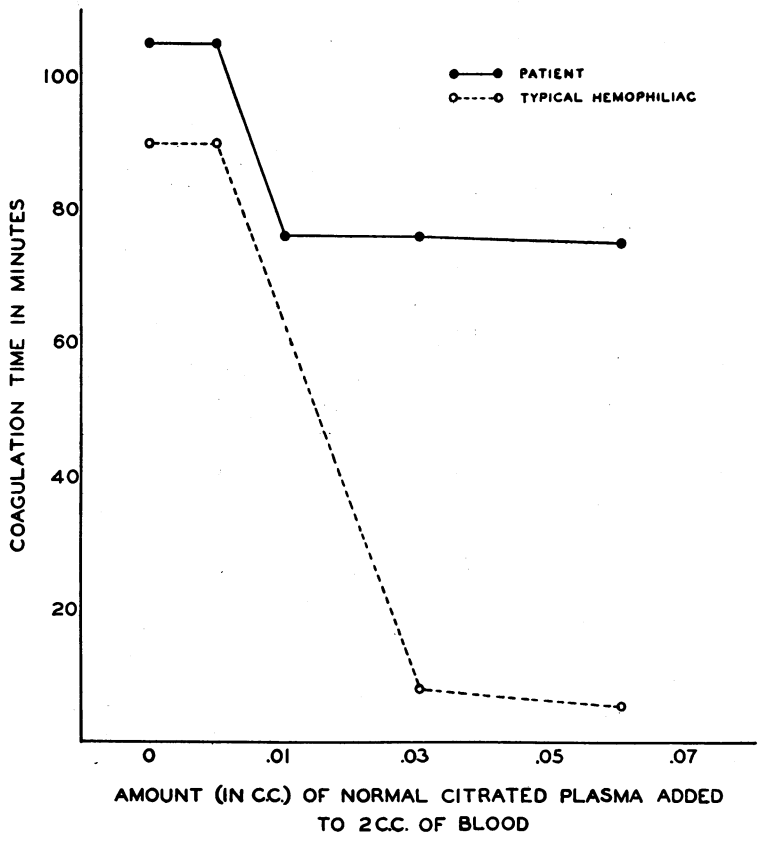
The plasma was dissolved in normal saline solution in an amount such that 0.5 c.c. of the solution was equivalent to approximately 1.0 c.c. of wet plasma. The total volume in each experiment for coagulation time was 2.0 c.c. — i.e., normal blood was added to bring the volume up to 2.0 c.c. No anti-coagulant was used in obtaining the plasma.

of normal blood was observed. Chart 2 shows that the patient's blood markedly prolonged the coagulation time of normal blood, whereas the blood of a true hemophiliac had no such effect. Blood from three other true hemophiliacs showed the same effect as given for the one hemophiliac in the chart.

Third, the possibility of the anti-coagulant being anti-thrombin was answered by testing the coagulation time of the plasma of the patient and that of normal subjects in the presence of purified thrombin. Table 2 gives the results of these studies. It is seen that the patient's plasma coagulated as rapidly in the presence of purified thrombin as did normal plasma. Hence, anti-thrombin would seem to have been excluded.

CHART I

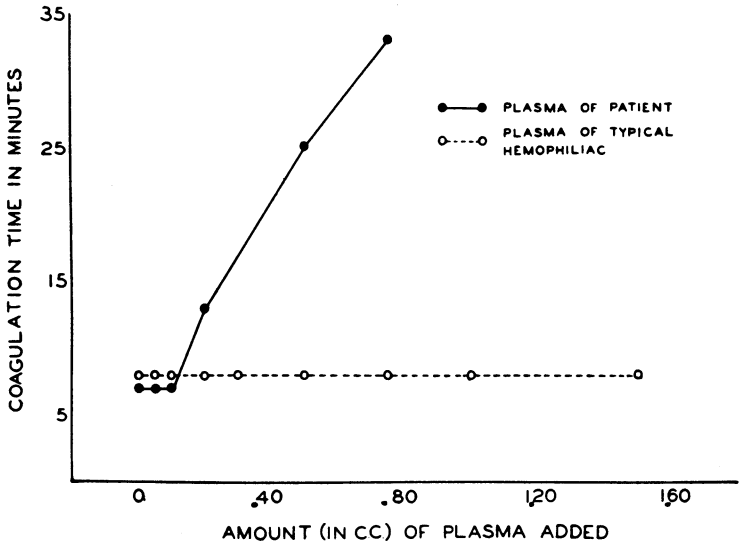
COMPARISON OF EFFECT OF NORMAL CITRATED PLASMA ON PATIENT'S BLOOD WITH THAT ON THE BLOOD OF A TYPICAL HEMOPHILIAC IN VITRO



Fourth, the effect of dried plasma and of ether extracted plasma was studied. Forty-five c.c. of the patient's plasma was dried, 4.3 gms. of dry plasma being obtained. Thus, each cubic centimeter of wet plasma was equivalent to 0.0956 gm. of dried plasma. When 4.3 gms. of dried plasma were extracted with ether for 8½ hours at 35° C., 1.19 gms. of residue were obtained. Approximately, 0.3824 gm. (equiva-

CHART 2

EFFECT OF PATIENT'S PLASMA ON THE COAGULATION TIME OF NORMAL BLOOD IN VITRO



IN EACH INSTANCE, NORMAL BLOOD WAS ADDED TO THE PLASMA SO AS TO BRING THE TOTAL VOLUME UP TO 2CC. THE PLASMA WAS OBTAINED BY CENTRIFUGING BLOOD FROM THE HEMOPHILIACS IN PARAFFINED TUBES.

lent to 4 c.c. plasma) of dried plasma was dissolved in 2 c.c. of normal saline solution. Then, 0.5 c.c. of this solution was put into each of 4 tubes and the effect on coagulation time of normal blood was determined. Table 3 shows the results obtained with the dried plasma and the ether extracted plasma. It will be seen that the anti-coagulant was not destroyed by drying or by ether extraction. As shown in Table 3, 0.25 c.c. of the redissolved ether extracted plasma prolonged the coagulation time of normal blood much more than 0.25 c.c. of the unextracted plasma. The probable explanation for this difference is the fact that the weights of the ether extracted plasma and unextracted dried plasma used in the final test solutions were approximately the same. Since the extracted plasma was lighter after extraction than

before, 0.25 c.c. of this plasma represented a larger volume of original wet plasma than 0.25 c.c. of the redissolved unextracted plasma.

The following additional characteristics of the anti-coagulant were established:

(1) It was not destroyed by a temperature of 37.5° C. for 30 hours; (2) It withstood a temperature of 5° C. for 30 hours; and (3) It did not pass through a collodion membrane when dialyzed with 0.85 per cent sodium chloride solution either when in oxalated or non-oxalated blood.

Finally, many experiments were done when protamine in amounts varying from 0.001 mg. to 0.15 mg. was added to tubes containing 1.5 c.c. of normal whole blood and 0.5 c.c. of the patient's plasma. In no instance, were we able to shorten the coagulation time of the patient's blood nor eliminate the anti-coagulant effect of the patient's plasma by such use of protamine. If the theory of Chargoff and Olson[1] and of Jaques and his associates[2] is correct, this eliminates heparin as the anti-coagulant.

Discussion.

Our findings as regards the anti-coagulant are almost identical with those of Lozner, Joliffe and Taylor.[3] However, neither they nor we have been able to carry the work far enough to actually identify the anti-coagulant and it is even conceivable that we are dealing with different substances. That the anti-coagulant was not heparin would seem to be established. The possibility of certain of the sulphur compounds such as cysteine, taurocholic acid and taurine being responsible for the anti-coagulant effect must be considered since these substances have been shown by Sterner and Medes[6] to act as anti-coagulants. We have made no observations which will allow us to make any statements in this regard.

One important question arises as to whether we were dealing with a true case of hemophilia. We believe that we were for the following reasons: 1. The family history is typical; 2. The clinical history of the patient is typical; 3. He has always shown a prolonged coagulation time; 4. Prothrombin, calcium, fibrinogen and platelet values have been normal; 5. The Quick test for recalcification of plasma has been positive for hemophilia. Against the diagnosis of hemophilia should be mentioned the failure of the patient's blood to show as

marked a diminution in the coagulation time following transfusion with normal blood as does the usual case and the marked prolongation of the coagulation time of normal blood in vitro in the presence of the patient's plasma. However, there was some diminution in the coagulation time of the patient's blood when normal blood was given by transfusion so that some effect was present. If this is a case of hemophilia, it constitutes the first instance of which we are aware, when an anti-coagulant has been demonstrated in the blood of a hemophiliac.

CONCLUSIONS.

A 43-year-old male with the family and clinical history of hemophilia has been shown to have a circulating anti-coagulant in his blood. The exact nature of this anti-coagulant is not known. It would seem not to be heparin.

In view of the findings in this patient, it would seem to us advisable to have the coagulation time checked shortly after the administration of fresh normal blood to every case of hemophilia. If the coagulation time should not fall in the expected manner, further investigations should be made to rule out the presence of a circulating anti-coagulant.

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